

We Claim:

1. A method for creating an array of probes comprising the steps of:
- a) synthesizing a first set of nucleic acids each comprising a constant sequence of length C at a 3'-terminus and a random sequence of length R at a 5'-terminus;
  - b) synthesizing a second set of nucleic acids each comprising a sequence complementary to the constant sequence of each of the first nucleic acid; and
  - c) hybridizing the first set with the second set to create the array.
2. The method of claim 1 wherein the nucleic acids of the first set are each between about 15-30 nucleotides in length and the nucleic acids of the second set are each between about 10-25 nucleotides in length.
3. The method of claim 1 wherein C is between about 7-20 nucleotides and R is between about 3-5 nucleotides.
4. The method of claim 1 wherein the array comprises about  $4^R$  different probes.
5. The method of claim 1 wherein the array is fixed to a solid support and the solid support is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and chips.
6. An array of probes created by the method of claim 1.
7. A method for creating an array of probes fixed to a solid support comprising the steps of:

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- a) synthesizing a first set of nucleic acids each comprising a constant sequence of length C at a 3'-terminus and a random sequence of length R at a 5'-terminus;
  - b) fixing the first set to the solid support;
  - c) synthesizing a second set of nucleic acids each comprising a sequence complementary to the constant region of the first set; and
  - d) hybridizing the nucleic acids of the first set with the second set to create the array.

8. A method for creating an array of probes comprising the steps of:

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- a) synthesizing an array of single-stranded nucleic acids each containing a constant sequence at the 3'-terminus, another constant sequence at the 5'-terminus, and a random internal sequence of length R flanked by the cleavage sites of a restriction enzyme;
  - b) synthesizing an array of primers each complementary to a portion of the constant sequence of the 3'-terminus, hybridizing the two arrays together to form hybrids;
  - c) extending the sequence of each primer by polymerization using a sequence of the nucleic acid as a template; and
  - d) cleaving the extended hybrids with the restriction enzyme to form an array of probes with a double-stranded portion at one terminus, a single-stranded portion containing the random sequence at the opposite terminus.

9. The method of claim 8 wherein the nucleic acids are each between about 10-50 nucleotides in length.

25 10. The method of claim 8 wherein R is between about 3-5 nucleotides in length.

11. The method of claim 8, wherein the restriction enzyme is selected from the group consisting of restriction enzymes which produce 5'-overhangs and restriction enzymes which produce 3'-overhangs.
12. The method of claim 8, wherein the array of probes is fixed to a solid support and the solid support which is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and chips.
13. An array of probes created by the method of claim 8.
14. A method for creating an array of probes comprising the steps of:
- synthesizing an array of single-stranded nucleic acids each containing a constant sequence at the 3'-terminus, another constant sequence at the 5'-terminus, and a random internal sequence of length R flanked by the cleavage sites of a restriction enzyme;
  - synthesizing an array of primers with a sequence complementary to the constant sequence at the 3'-terminus;
  - hybridizing the two arrays together to form hybrids;
  - enzymatically extending the primers using the nucleic acids as templates to form full-length hybrids;
  - cloning the full-length hybrids into vectors;
  - amplifying the cloned sequences by multiple polymerase chain reactions; and
  - cleaving the amplified sequences with the restriction enzyme to form the array of probes with a double-stranded portion at one terminus and a single-stranded portion containing the random sequence at the opposite terminus.
15. The method of claim 14, wherein the array of probes have 5'- or 3'-overhangs.

16. The method of claim 14, wherein the array of probes is fixed to a solid support and the solid support is selected from the group consisting of plastics, ceramics, metals, resins, polymers, films, gels, membranes and chips.

17. An array of probes created by the method of claim 14,

5 18. A method for detecting a nucleic acid in a biological sample comprising the steps of:

- a) creating an array of probes fixed to a solid support according to the method of claim 7;
- b) labeling the nucleic acid of the biological sample with a detectable label;
- 10 c) hybridizing the labeled nucleic acid to the array; and
- d) detecting the sequence of the nucleic acid from a binding pattern of the label on the array.

19. A method for identifying a target nucleic acid in a biological sample comprising the steps of:

- 15 a) creating an array of probes fixed to a solid support according to the method of claim 7;
- b) labeling the target of the biological sample with a detectable label;
- c) hybridizing the labeled target to the array; and
- d) identifying the target from a binding pattern of the label on the array.

20. The method of claim 19, wherein the detectable label is selected from the group consisting of radioisotopes, stable isotopes, enzymes, fluorescent and luminescent chemicals, chromatic chemicals, metals, electric charges, and spatial chemicals.

21. The method of claim 19 wherein the nucleic acid identified is selected from the group consisting of nucleic acids derived from viruses, bacteria, parasites, fungi and yeast.
22. The method of claim 19 wherein the binding pattern is a nucleic acid fingerprint.
23. A diagnostic aid for detecting a target nucleic acid in a biological sample comprising the array of claim 19, a solid support on which the array is fixed, a detectable label, and the biological sample.
24. The method of claim 19 wherein the biological sample is selected from the group consisting of samples of animal tissue, environmental substances, and manufacturing products and by-products.
25. The method of claim 24 wherein the animal tissue is obtained from a human.
26. The method of claim 19 further comprising the step of purifying the target nucleic acids identified.
27. A method for replicating an array of single-stranded probes on a solid support comprising the steps of:
- synthesizing an array of nucleic acids each comprising a constant sequence of length C at a 3'-terminus and a random sequence of length R at a 5'-terminus;
  - fixing the array to a first solid support;
  - synthesizing a set of nucleic acids each comprising a sequence complementary to the constant sequence;
  - hybridizing the nucleic acids of the set with the array;

- e) enzymatically extending the nucleic acids of the set using the random sequences of the array as templates;
- f) denaturing the set of extended nucleic acids; and
- g) fixing the denatured nucleic acids of the set to a second solid support to create the replicated array of single-stranded probes.

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28. The method of claim 27 wherein the nucleic acids of the set are conjugated with biotin and the second solid support comprises streptavidin.

29. The method of claim 27 wherein the nucleic acids of the array are between about 15-30 nucleotides in length and the nucleic acids of the set are between about 10-25 nucleotides in length.

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30. The method of claim 27 wherein C is between about 7-20 nucleotides and R is between about 3-5 nucleotides.

31. The method of claim 27 wherein the solid support is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and chips.

32. The method of claim 27 wherein the nucleic acids of the set are enzymatically extended with a DNA polymerase and one or more deoxynucleotide triphosphates.

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33. The method of claim 27 wherein denaturing is performed with heat, alkali, organic solvents, binding proteins, enzymes, salts or combinations thereof.

34. A replicated array of single-stranded probes made by the method of claim 27.

35. The method of claim 27, further comprising the step of hybridizing the replicated array with a second set of nucleic acids complementary to the constant sequence of the replicated array to create a double-stranded replicated array.

36. A replicated array of double-stranded probes made by the method of claim 35.

37. A method for creating a probe comprising the steps of:

- a) synthesizing a plurality of first nucleic acids and a plurality of second nucleic acids comprising a random terminal sequence and a sequence complementary to a sequence of the first nucleic acids;
- b) hybridizing the first nucleic acids with the second to form partial duplexes;
- c) hybridizing a target nucleic acid to the partial duplexes;
- d) ligating the hybridized target to the first nucleic acid of the partial duplexes;
- e) isolating the second nucleic acid from the ligated duplexes; and
- f) synthesizing a plurality of third nucleic acids each complementary to the constant sequence of the second nucleic acid and hybridizing the third nucleic acids with the isolated second nucleic acids to create a probe.

38. The method of claim 37 wherein the first nucleic acids are each between about 15-25 nucleotides in length and the second nucleic acids are each between about 20-30 nucleotides in length.

39. The method of claim 37 wherein the target is hybridized to the partial duplexes under a single set of hybridization conditions.

40. The method of claim 39 wherein the hybridization conditions comprise a temperature of between about ~~22-37°C~~ <sup>22-37°C</sup>, a salt concentration of between about 0.05-0.2 M, and a time period of between about 1-14 hours.

41. The method of claim 37, wherein a double-stranded portion of the partial duplex contains an enzyme recognition site.

42. A probe created by the method of claim 37,

43. The probe of claim 42, which is fixed to a solid support and the solid support is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and chips.

44. A diagnostic aid for the detection of a target nucleic acid in a biological sample comprising the probe of claim 42, a solid support on which the probe is fixed, a detectable label, and the biological sample.

45. A method for creating a probe comprising the steps of:

- a) synthesizing a plurality of first nucleic acids and a plurality of second nucleic acids each comprising a random terminal sequence and a sequence complementary to the sequence of the first nucleic acids;
- b) hybridizing the first nucleic acids with the second nucleic acids to form partial duplexes having a double-stranded portion and a single-stranded portion with the random sequence within the single-stranded portion;
- c) hybridizing a target nucleic acid to the partial duplexes;
- d) ligating the hybridized target to the first nucleic acid of the partial duplex;
- e) hybridizing the ligated target with a set of oligonucleotides comprising random sequences;
- f) ligating the hybridized oligonucleotide to the second nucleic acid;
- g) isolating the oligonucleotide ligated second nucleic acid; and
- h) synthesizing another plurality of first nucleic acids and hybridizing the first nucleic acids with the isolated second nucleic acid to create the probe.



46. The method of claim 45, wherein the first nucleic acids are each between about 15-25 nucleotides in length, the second nucleic acids are each between about 20-30 nucleotides in length, and the oligonucleotides are each between about 4-20 nucleotides in length.

5 47. The method of claim 45, wherein the target is hybridized to the partial duplexes under a single set of hybridization conditions.

48. The method of claim 45, wherein the hybridization conditions comprise a temperature of between about ~~22-37°C~~ <sup>28-37°C</sup>, a salt concentration of between about 0.05-0.2 M, and a time period of between about 1-14 hours.

10 49. The method of claim 45 wherein the partial duplexes contain an enzyme recognition site.

50. A nucleic acid probe created by the method of claim 45.

51. The nucleic acid probe of claim 50, which is fixed to a solid support selected from the group consisting of plastics, ceramics, metals, resin, gels, membranes and chips.

15 52. A diagnostic aid for the detection of a target nucleic acid in a biological sample comprising the probe of claim 45, a solid support on which the probe is fixed, a detectable label, and the biological sample.

53. A method for creating a probe comprising the steps of:

- a) synthesizing a plurality of first nucleic acids and a plurality of second nucleic acids comprising a random terminal sequence and a sequence complementary to a sequence of the first nucleic acid;

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- b) hybridizing the first nucleic acids to the second nucleic acids to form partial duplexes having a double-stranded portion and a single-stranded portion with the random nucleotide sequence within the single-stranded portion;
  - c) hybridizing a target nucleic acid to the partial duplexes;
  - d) ligating the hybridized target to the first nucleic acid of the partial duplex;
  - e) enzymatically extending the second nucleic acid using the target as a template;
  - f) isolating the extended second nucleic acid; and
  - g) synthesizing another first nucleic acid and hybridizing the first nucleic acid with the isolated and extended second nucleic acid to create a probe.
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54. The method of claim 53 wherein the first nucleic acids are each between about 15-25 nucleotides in length and the second nucleic acids are each between about 20-30 nucleotides in length.

15 55. The method of claim 53 wherein the target is hybridized to the partial duplexes under a single set of hybridization conditions.

B 56. The method of claim 55 wherein the hybridization conditions comprise a temperature of between about 22-37°C, a salt concentration of between about 0.05-0.2 M, and a time period of between about 1-14 hours.

20 57. The method of claim 53 wherein the double-stranded portion contains an enzyme recognition site.

58. The method of claim 53 wherein the target nucleic acid is obtained from a biological sample selected from the group consisting of samples of animal tissue, environmental substances, and manufacturing products and by-products.

59. A nucleic acid probe created by the method of claim 53.

60. The nucleic acid probe of claim 59 which is fixed to a solid support and the solid support is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and chips.

5 61. A diagnostic aid for the detection of a target nucleic acid in a biological sample comprising the nucleic acid probe of claim 59, a solid support on which the probe is fixed, a detectable label and the biological sample.

10 62. An array of  $4^R$  different nucleic acid probes wherein each probe comprises a double-stranded portion of length D, a terminal single-stranded portion of length S, and a random nucleotide sequence within the single-stranded portion of length R.

63. The array of claim 62, wherein D is between about 3-20 nucleotides and S is between about 3-20 nucleotides.

15 64. The array of claim 62 which is fixed to a solid support wherein the solid support is selected from the group consisting of plastics, ceramics, metals, resins, gels, membranes and two-dimensional and three-dimensional matrices.

Add C3

Add D6

Add G1